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Review

Towards a better understanding of cognitive behaviors regulated by gene expression downstream of activity-dependent transcription factors

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ABSTRACT

In the field of molecular and cellular neuroscience, it is not a trivial task to see the forest for the trees, where numerous, and seemingly independent, molecules often work in concert to control critical steps of synaptic plasticity and signalling. Here, we will first summarize our current knowledge on essential activity-dependent transcription factors (TFs) such as CREB, MEF2, Npas4 and SRF, then examine how various transcription cofactors (TcoFs) also contribute to defining the transcriptional outputs during learning and memory. This review finally attempts a provisory synthesis that sheds new light on some of the emerging principles of neuronal circuit dynamics driven by activity-regulated gene transcription to help better understand the intricate relationship between activity-dependent gene expression and cognitive behavior.

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1. Introduction

Over the last 25 years or so, it has become evident that activity-dependent gene expression plays essential roles in not only globally controlling memory persistence, but most importantly also to define and determine the genesis and decay of local memory traces. Significant cognitive deficits were associated with aberrant functions of activity-dependent transcription factors (TFs) both in mice and in humans (West & Greenberg, 2011). Consistent with this notion, many of their target genes that are strongly induced by neuronal activity (often referred to as immediate early genes (IEGs)) show high utility as biomarkers of activated neurons (Okuno, 2011). Conversely, disturbed IEG expression correlates with cognitive disorders under many pathophysiological states (Cohen & Greenberg, 2008). Recent evidence has further revealed that activity-regulated TFs may not just function as molecular switches that activate IEG promoters to control expression of downstream genes. The state of activity-regulated TFs might dynamically allocate memory within a functional neuronal circuit (Liu et al., 2012; Mayford, 2014; Yiu et al., 2014), and this may be a mechanism to assign the active neuronal ensemble that defines a memory engram within a given brain area (Silva, Zhou, Rogerson, Shobe, & Balaji, 2009). A number of recent IEG promoter-based labeling and cell manipulation studies support this idea (Kawashima, Okuno, & Bito, 2014). However, key conceptual advances sometimes fail to draw public attention, especially when early critical findings are fragmented and presented in many different and controversial pieces.

Here we will aim to first summarize recent advances in the research of individual activity-dependent TFs (Table 1), then will present an outlook of the technical advances that recently provide a new framework for understanding activity-dependent gene expression in the context of cognitive behavioral neuroscience.

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Table 1
Brief summary of basic properties and recent findings on transcription factors/cofactors related to cognitive functions described in the Sections 1–5 of the text.

Activity-dependent transcription factor	Target sequence	Cofactor	Localization and activation	Downstream genes (e.g., Cognitive effects)		References
CREB	CRE	CRTC1, CBP	Constitutively bound to target Ca^{2+}-dependent phosphorylation	*c-fos, Arc, BDNF, Egr-1*	Memory enhancer	Josselyn et al. (2001) Han et al. (2007)
MEF2	MRE	CBP	Constitutively nucleus	*c-fos, Arc*	Memory repressor	Flavell et al. (2008) Rashid et al. (2014)
Npas4	Not determined		Ca^{2+}-dependent Npas4 induction and DNA binding	*BDNF*	Regulation of inhibitory/ excitatory circuits	Lin et al. (2008) Spiegel et al. (2014)
SRF	SRE	TCL, MKL1	Constitutively bound to the target	*c-fos, Arc, Egr-1*	LTP induction and maintenance/LTD maintenance	Ramanan et al. (2005) Posern and Treisman (2006)
DREAM / KChIP3	DRE (Shared with CREB/ CREM binding sites)		Ca^{2+} binding dependent release form the promoter	*c-fos, Npas4, Mef2c*	DG-enriched LTP enhancer	Carrion et al. (1999) Mellstrom et al. (2014)
MeCP2	Methyl CpG		Ca^{2+}-triggered phosphorylation dependent release from the promoter	*CREB, BDNF, Sst, Mef2c*	causative gene for *Rett* syndrome (a type of autism)	Guy et al. (2011) Chahrour et al. (2008)

2. Molecular biology of a leading neuronal activity-dependent transcription factor that facilitates memory formation: CREB

Ca^{2+}/cyclic AMP response element-binding protein (CREB) is widely expressed in the brain and other organs, throughout development, and is critical in many forms of cognitive behavior, including memory formation and allocation (Bourtchuladze et al., 1994; Gruart, Benito, Delgado-Garcia, & Barco, 2012; Han et al., 2007; Kida et al., 2002; Silva, Kogan, Frankland, & Kida, 1998; Silva et al., 2009; Suzuki et al., 2011; Yin et al., 1994; Zhou et al., 2009). CREB is further implicated in many brain functions such as neuronal survival, proliferation, ischemia, circadian clock, plasticity, and feeding behavior (Gau et al., 2002; Mantamadiotis et al., 2002; Martin & Kandel, 1996; Nonaka, 2009). Despite its overwhelming importance, the pursuit of what defines its regional and downstream specificity has remained rather elusive.

Essentially, CREB localizes in the nucleus, binds to a cAMP-responsive element (CRE) sequence, -TGACGTCA-, and is activated downstream of various kinase cascades stimulated by cAMP, Ras, and/or Ca^{2+} signaling, all of which converge on the phosphorylation of Ser^{133} residue (Arthur et al., 2004; Bito et al., 1996; Finkbeiner et al., 1997; Ginty et al., 1994; Gonzalez & Montminy, 1989; Hardingham et al., 1997; Impey et al., 1996; Naqvi et al., 2014; Sheng et al., 1991). CREB associates with well-known co-factors, CREB binding protein (CBP) and its homologue p300 (Goodman & Mandel, 1998). These bind to p-Ser^{133} of CREB in response to external stimuli and then recruit the RNA polymerase II-containing transcription initiation complex, while they also acetylate histone to loosen the nearby chromatin structure. CBP chromatin immunoprecipitation (ChIP)-seq data revealed that upon stimulation CBP-binding sites on the whole genome increased from 1000 to ~28,000 (Kim et al., 2010). CBP/p300 were shown to have possible crosstalk with TFs other than CREB (Ramos et al., 2010). These lines of evidence suggest that CBP may not be a CREB-specific cofactor, but rather a common scaffold for RNA polymerase II at enhancers and promoters. This study further indicated that CBP and p300, seemingly redundant transcriptional cofactors (TcoFs), displayed some extent of specificity (Ramos et al., 2010).

Recent evidence indicated that CREB phosphorylation at Ser^{133} may not be the sole CREB activation mechanism in all CREB-regulated genes. Comparative CREB ChIP-chip analysis in different cell types revealed that out of ~4000 gene promoters occupied by CREB, transcription of only ~100 genes was actually up-regulated in response to forskolin, a cAMP production stimulant. Furthermore, distinct cell types showed differential induction of alternate sets of ~100 genes although there was no specificity at the level of either CREB expression or p-CREB status (Zhang et al., 2005). Thus, novel mechanisms that determine cellular specificity of CREB activation await to be identified.

Coincidently, CREB-regulated transcriptional co-activator (CRTC) or transducer of regulated CREB activity (TORC) was identified as a co-factor that binds the bZIP domain of CREB, and activate CREB in a manner that acts in parallel to CREB phosphorylation (Altarejos & Montminy, 2011; Conkright et al., 2003). Of three known isoforms, CRTC1-3, CRTC1 is the dominant TcoF isoform expressed in the nervous system (Watts, Sanchez-Watts, Liu, & Aguilera, 2011). CRTC1 translocates from cytosol to nucleus in response to synaptic activity (Ch'ng et al., 2012) and its overexpression in the hippocampus results in enhanced contextual memory (Nonaka et al., 2014; Sekeres et al., 2012). Furthermore, CRTC1 regulates dendritic morphology (Li, Zhang, Takemori, Zhou, & Xiong, 2009). *In vivo* studies suggested that CRTC1 promotes transcription of CRE-dependent genes such as *c-fos*, *BDNF*, *Arc* (also termed as *Arc/Arg3.1*) and *Egr-1*(also termed as *zif-268*), and revealed interesting brain region-specificity in regulation of task-dependent CRTC1 nuclear shuttling (Nonaka et al., 2014). Therefore, CRTC1 might play a role in determining CREB specificity at both the circuit and the chromosomal levels in the brain.

3. An activity-dependent transcription factor that represses memory: MEF2

Myocyte enhancer factor-2 (MEF2) is a TF that has originally been characterized for its role during muscle development. It is now evident, however, that MEF2 regulates neuronal development and survival. MEF2 has four isoforms, MEF2A-D, which are expressed in a brain-region specific manner (Rashid, Cole, & Josselyn, 2014). It constitutively localizes in the nucleus and binds to MRE. The consensus motif of MRE appears more tolerant in brain (underlined sequence of TGTTACT(A/t)(a/t)AAATAGA(A/t)) than as found in the muscle, which might account for how MEF2 induces distinct sets of genes in those tissues (Andres, Cervera, & Mahdavi, 1995). Transcriptome analyses using MEF2-deficient cells identified many downstream genes including *c-fos* and *Arc* (Flavell et al., 2008).

An emerging view for the role of MEF2 in cognition in the mature brain is that MEF2 may act as a repressor for memory (Rashid et al., 2014). MEF2 may gate memory formation as it undergoes task-dependent phosphorylation and degradation. MEF2 also negatively regulates spine density (Cole et al., 2012;

Flavell et al., 2008; Vetere, Restivo, et al., 2011). While a mild *NSE*-promoter driven expression of an active form of MEF2 (MEF2-VP16) in transgenic mice showed little effect in a fear conditioning test, an HSV-mediated, temporally and spatially restricted, overexpression of MEF2-VP16 revealed suppression of fear and spatial memory, while active MEF2 also inhibited the task-dependent increase in spine density (Cole et al., 2012). Conversely, blocking MEF2 function by HSV-mediated expression of a dominant negative form of MEF2 facilitated memory formation (Cole et al., 2012). These lines of experiments suggest a technical advantage for a virus-mediated and local expression strategy in delineating causality.

This negative regulation for spatial memory and fear memory might be mediated at least in part by Arc, as Arc overexpression was previously shown to facilitate AMPAR internalization (Chowdhury et al., 2006; Okuno et al., 2012; Shepherd et al., 2006). However, *Arc* is also upregulated downstream of CREB and SRF, both of which are believed to correlate with enhanced memory. Treatment with memory-enhancing drugs also commonly triggers induction of IEGs that include known MEF2 target genes. Therefore, further investigation is clearly needed to tease apart the critical contribution of MEF2, vis-à-vis of the TF network that controls transcriptional signal processing underlying memory formation (Rashid et al., 2014).

4. A new kid on the block: Npas4

Npas4 is a bHLH-type TF that is stringently induced by Ca^{2+}-influx driven voltage-gated channels, but not by neurotrophic factors or cAMP increases in neurons (Lin et al., 2008). Hence, Npas4 itself is an IEG, which is induced by synaptic activity both in excitatory and inhibitory neurons (Spiegel et al., 2014) as well as during ischemia and seizure (Maya-Vetencourt, 2013). Consistent with the fact that the Npas4 is activity-induced, genome-wide ChIP-seq confirmed the absence of Npas4 binding in the activity-silenced state, in sharp contrast to CREB and SRF, a large portion of which are constitutively bound to their targets (Kim et al., 2010). The function of Npas4 is associated with formation and maintenance of GABAergic inhibitory synapses as part of a homeostatic excitability regulation mediated in part through its direct target *BDNF* (Lin et al., 2008; Spiegel et al., 2014). Furthermore, Npas4 in hippocampal CA1 was revealed to contribute to a specific facilitation of inhibitory synapse formation around the soma, while inhibitory synapses were reduced in the apical dendrites (Bloodgood, Sharma, Browne, Trepman, & Greenberg, 2013). Such Npas4-mediated rewiring will have a significant impact on information processing in the CA1 pyramidal neurons, as an increase in somatic inhibitory inputs will reduce cell firing, while a reduction in dendritic inhibitory inputs will affect gating of local inputs.

Another region-specific behavioral contribution was reported for Npas4; a virus-mediated local deletion in CA3, but not in CA1, impaired fear memory (Ramamoorthi et al., 2011). Therefore, Npas4 may mediate a region-specific rewiring and plasticity processes within the hippocampus.

Such region specificity and cell type specificity (inhibitory and excitatory neurons) of TFs'/TcoFs' functions has often been overlooked in classical global genetic manipulations but is now being probed using local virus injection and neuronal subtype-specific Cre transgenic approaches (Spiegel et al., 2014).

5. A critical role for SRF in circuit rewiring and plasticity

Serum responsive factor (SRF) is a MADS domain-containing TF that binds to the sequence, $CC(A/T)_6GG$ (called CArG box) within an SRE and plays important roles in mesoderm and muscle development, anti-apoptotic processes, neuronal migration and synaptic plasticity. Its downstream genes in neurons include a large body of IEGs, such as *c-fos*, *Arc* and *Egr-1*, just to name a few. SRF activation is mainly dependent on its TcoFs, Elk1, a member of the ternary complex factor (TCF), and Megakaryoblasic Acute Leukemia-1 (MAL/MKL1), a member of the myocardin-related transcription factor (MRTF) (Besnard, Galan-Rodriguez, Vanhoutte, & Caboche, 2011; Kalita, Kuzniewska, & Kaczmarek, 2012). TCF and MAL share the same binding region on the DNA binding site of SRF. Thus, the binding of TCF and MAL occurs in a mutually exclusive manner (Posern & Treisman, 2006). Certain SRE elements appeared to be biased towards preferential binding of either SRF/TCF or SRF/MAL complexes, implying the specific contribution of either of the cofactors for the promoters. This alternative cofactor system might contribute to the tissue/region- and/or developmental stage-specific induction of some SRF-dependent genes. SRF has distinct roles in glial cells and neurons. SRF is implicated in glial specification (Lu & Ramanan, 2012) and SRF in astrocytes may modulate ocular dominance plasticity (Paul et al., 2010). In neurons, SRF is not essential for survival but is critical for induction and maintenance of LTP (Ramanan et al., 2005) and maintenance of LTD (Etkin et al., 2006; Smith-Hicks et al., 2010). TCF activation via MAPK-ERK1/2 pathway is well established, but it has been shown that synaptic activity is also activating MAL through ERK1/2 (Kalita, Kharebava, Zheng, & Hetman, 2006). MAL is regulated downstream of the RhoA signalling pathway. G-actin depletion releases MAL to translocate to the nucleus (Tabuchi et al., 2005). *Srf* conditional knockout ($Srf^{-/-}$; *Camk2a-iCre*) revealed severe defects in neuronal axon branching, dendrites, and spine morphology (Stritt & Knoll, 2010). It is as yet not clear whether SRF may also regulate activity-dependent circuit rewiring in the adult brain in association with plasticity regulation in a manner similar to the case of Npas4.

6. Important co-factors that modulate IEG transcription: DREAM and MeCP2

DREAM (downstream regulatory element antagonist modulator or antagonist modulator of DRE sites), also termed as KChIP3 or calsenilin, is a Ca^{2+}-regulated transcription modulator that represses transcription when bound to target DRE sites (-GTCA- or inverted sequence, -TGAC-). It is unique in that DREAM is directly regulated by Ca^{2+} through its four EF hands. Upon Ca^{2+} binding, DREAM unbinds from DRE sites, thus removing the inactivation of transcription (Carrion, Link, Ledo, Mellstrom, & Naranjo, 1999). This protein is specifically enriched in the hippocampal dentate gyrus (DG). Upon contextual fear conditioning test, it translocates from the plasma membrane Kv channels, to the nucleus in 6 h to suppress target gene expression. The dissociation of DREAM/KChIP3 from Kv channels will contribute to enhanced A-currents, which in turn alter general excitability and LTP induction property. The negatively regulated targets include *Prodynorphin*, *c-fos*, *ICER*, and *CREM* (Ledo et al., 2000). Presumably because of compensation by other KChIP isoforms, a general DREAM knockout barely displayed any neuronal phenotypes. DREAM has been reported to associate with the KID domain of CREB or some CREM isoforms, and thus interfere with CREB-CBP binding. Upon Ca^{2+} binding, DREAM is released from CREB/CREM, while in parallel, CREB phosphorylation cause a conformational change that strongly favors CBP binding (Ledo, Kremer, Mellstrom, & Naranjo, 2002). Although the exact details of this pathway are yet to be determined, DREAM might serve as a DG-specific regulator for Ca^{2+} signalling to CREB-CBP complex formation. Consistent with this idea, DREAM knockouts show enhanced contextual fear memory (Alexander et al., 2009), and transgenic mice expressing a dominant active DREAM (daDREAM) showed strong deficits in water-

maze, a spatial memory task and slight deficit in active avoidance test, all of which involves the hippocampus (Mellstrom et al., 2014). In keeping with evidence that *Npas4* and *BDNF* are regulated by DREAM, daDREAM transgenic mice showed downregulation of GABAergic synapses and enhanced LTP in DG (Mellstrom et al., 2014).

MeCP2 is an epigenetic repressor of transcription that binds to the methylated CpG, within a large number of promoters including that of the *BDNF* gene. Mutations in the *MeCP2* gene are causative for a majority of familial cases of the Rett syndrome, although the exact cell type/brain region in which MeCP2 dysregulation is most critical still remains debated (Castro, Mellios, & Sur, 2013; Guy, Cheval, Selfridge, & Bird, 2011). Upon Ca^{2+}-triggered phosphorylation, MeCP2 unbinds from its target locus and from co-repressor, NCoR, thus enabling restart of transcription (Cohen et al., 2011; Ebert et al., 2013). Surprisingly, it has been reported that MeCP2 may have a distinctive region-specific role in the hypothalamus where it acts as an activator for most of the target genes, partially collaborating with CREB on some (e.g. somatostatin) but not all CRE-dependent genes (Chahrour et al., 2008). In this context, CREB itself is a positive target of MeCP2.

7. Taking advantage of technical breakthroughs in molecular virology to address the functional specificity of activity-dependent transcription factors *in vivo*

An exciting new development in the field of activity-dependent TFs is the growing availability of localized transcriptome data (Benito & Barco, 2014) and of new viral vector tools (Dreyer, 2011; Varenika et al., 2009). The former enables the identification of critical brain regions of transcriptional dysregulation, while the latter allows critical manipulation of transcriptional mechanisms with high spatial and temporal precision.

Virus-mediated brain region-specific interrogative approaches have proven to be invaluable in complementing conventional global transgenic approaches (simplified overview summarized in Fig. 1A). The combination of Cre-transgenic lines with virus-mediated expression cassettes, for example, currently enables the most sophisticated degree of cell-type and regional specificity required for circuit mapping. However, with improved protocols for high-titer viral vectors (HSV, AAV, or lentivirus), it has become possible to study behavioral phenotypes for specific gene knockdown/knockout or overexpression of a gain-of-function/dominant loss-of-function mutants of any transcriptional regulators (Adachi, Barrot, Autry, Theobald, & Monteggia, 2008; Berton et al., 2006; Han et al., 2007; Josselyn et al., 2001) (Fig. 1, Table 2).

In certain cases, proper selection of virus (and serotypes) and tissue-/celltype-specific promoters may be necessary for designing experiments. HSV, lentivirus, and AAV-mediated gene manipulation achieves different expression time courses and spatial spreads within the brain. HSV will express the proteins of interests within a few days after injection, then cease the expression in a week, while lentivirus and AAV-mediated expression will gradually increase and last onwards (Table 2 for references). Due to the particle size of the virus, AAV will generally spread in a larger area than lentivirus, but this feature also depends on the serotypes. These differences in temporal kinetics and spatial spreads of virus-mediated expression have implications on how behavioral phenotypes are to be tested (Table 2).

We should bear in mind that virus infection may not be achieved in all neurons in the area of virus spread and region-specific confinement and infectivity may usually be defined by a complex function of physical spread of the viral solution, the viral titer and the promoter strength. For loss-of function studies, injection of the Cre virus (that expresses Cre recombinase) into a floxed mice (in which a specific portion of a gene is flanked by loxP sequences and ready to be excised by Cre-dependent recombination) will create region-specific knockouts (Berton et al., 2006). In contrast, AAV or lentivirus or HSV-mediated shRNA expression will work as region-specific knockdowns. Careful interpretation of physiological and behavioral data is necessary to account for the potential residual effect due to insufficient infection efficiency and gene excision/knockdown efficiency. The mozaicism of viral expression might sometimes be desirable. For example, Bloodgood et al. (2013) used a combination of *Npas4* flox-mouse and Cre virus injection, which created a mosaic area within the virus-injected brain region and thus enabled them to simultaneously record electrically from both recombination-positive (thus KO) cells and juxtaposed negative (wild-type) cells.

8. Advanced tools for examining the causality of neuronal activity traces *in vivo*

To determine the causal role of activity-dependent gene expression, it is critical to tightly control the experimental time window for knockdown/knockout or gain-of-function gene expression, especially when those genetic modifications are to be restricted within certain phases of behavior tasks. Orthogonal recombinase systems, such as the Cre-loxP and the FLP-FRT systems, the tamoxifen-$CreER^{T2}$ and the doxycycline-TetON/OFF systems could prove to be very powerful, if the two gene activation events to be combined require a controlled temporal and spatial sequence. Improved designs of customized dual cassette vectors will particularly be helpful in such attempts (e.g. Kawashima et al., 2013).

Viral technologies (Jennings & Stuber, 2014) have been extensively exploited in elucidating the causal role of CREB in learning and memory. Based on the finding that forced expression of CREB in a subset of neurons at encoding will facilitate recruitment of a memory engram in CREB-positive neurons, several groups have now successfully modified memory performance by altering neuronal activity of these CREB-engram neurons by co-expression of effector proteins (Table 2) (Czajkowski et al., 2014; Han et al., 2007; Kim, Kwon, Kim, Josselyn, & Han 2014; Zhou et al., 2009). Furthermore, CREB-dependent recruitment of memory engrams was recapitulated by increasing excitability in a subset of neurons with virally expressed effectors such as dnKCNQ and the pharmacogenetic DREADD Gq system (Yiu et al., 2014). Independently, activity-dependent promoters were used to define the memory engram and to manipulate the activity of those engram neurons using optogenetic (Liu et al., 2012; Ramirez, Tonegawa, & Liu, 2013) or pharmacogenetic (Garner et al., 2012) effector proteins.

One attractive next-generation technology in the field of TF/TcoF research is the light-inducible transcriptional effectors, such as LITE (Konermann et al., 2013). While the potency of light-induced transcriptional activation still needs to be further optimized, this technique holds strong promise for future application in intact brain, using AAV for example, to trigger expression of specific set of IEGs with even higher temporal and spatial precision *in vivo*.

9. Cooperativity and combinatorial selectivity via crosstalk of activity-dependent transcription factors: towards a better understanding of activity-dependent gene expression in active neuronal ensembles

While numerous work has demonstrated that activity-dependent TFs play critical roles in cognition, it is yet not clear how co-activation of several activity-dependent TFs helps achieve

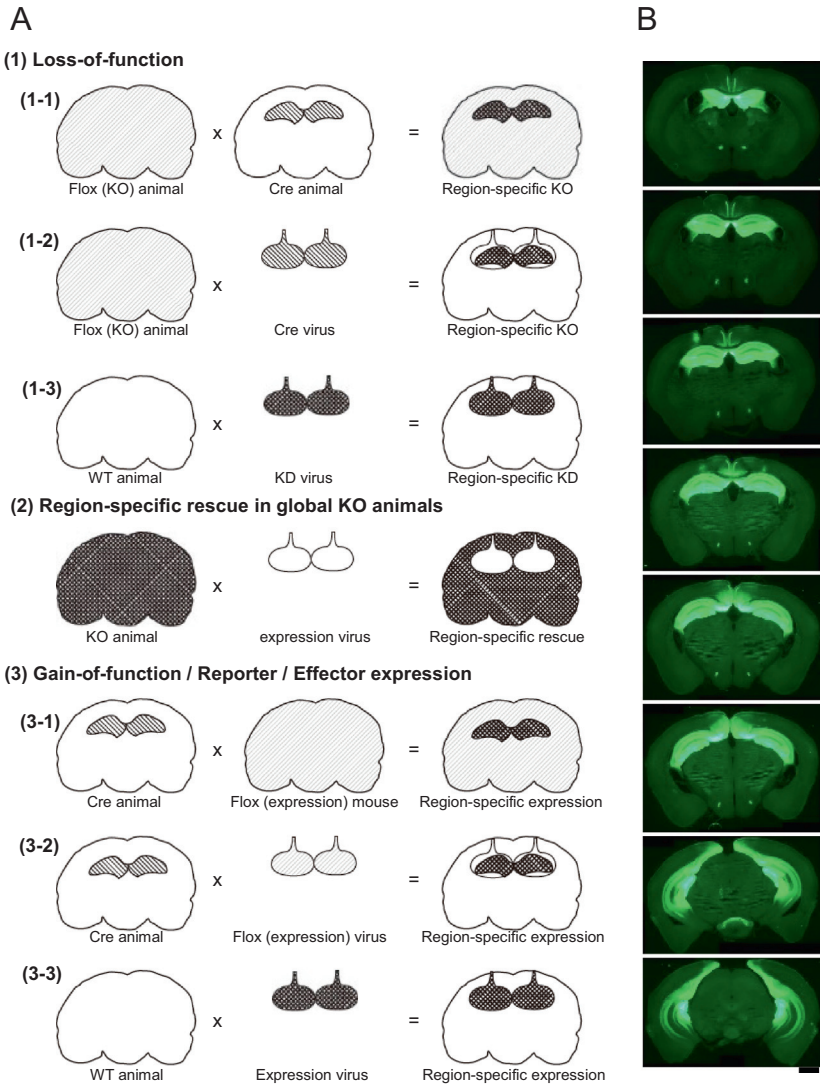

Fig. 1. (A) Simplified view of novel molecular virological strategies for brain-region specific genetic medication *in vivo*. (1-1) In loss-of-function studies, the currently standard method is to cross a conditional (floxed) KO animal with a Cre animal (which expresses Cre recombinase in a region- or population-specific manner). (1-2) Alternatively, we can use a viral expression vector encoding a Cre recombinase. This virus-mediated method is time-saving and sometimes even beneficial over Cre animal, in which the expression pattern of Cre may not be perfectly ideal. Use of low-titer Cre-virus results in the mosaic KO, which is sometimes suited for slice electrophysiology as well as morphological and immunohistochemical assays to test cell autonomy of the gene deletion effect. (1-3) Wild-type animal infected with knockdown-virus into the brain region of interest. (2) Targeted virus injection is also useful for the rescue experiment of global KO animals to verify that restoring the expression of the molecule in the particular region is sufficient to rescue the phenotypes. (3) In gain-of-function studies such as wild-type overexpression or active-form expression, Cre driver animal is crossed with conditional Flox-mediated gene expression line (3-1). (3-2) Injection of virus with Flox-mediated expression are also commonly used for localized gene expression. (3-3) Simply injecting gene-expression virus into wild-type animal is also a powerful way of region-specific genetic modification. The promoter of the expression cassette in the virus confers brain region- or celltype- specificity, when the size of the promoter is small enough to be inserted into a viral vector cassette. In experiments using activity-reporters or light-sensitive probes, such as GCaMP, or effector molecules, such as ChR2/NpHR/Arch, the same approaches as those used for gain-of-function are applicable. However, for better cell-type specificity, the combination of Cre animal and the Flox-mediated effector virus is more commonly used. (B) Serial section of AAV-GFP infection targeted to bilateral hippocampus in wild-type mouse. Scale, 1 mm. The virus-only approaches using wild-type animals in Fig. 1A, (1-3) and (3-3) are effective in selectively targeting distinct brain structures such as the hippocampus, the amygdala, or the olfactory bulb. In experienced hands and with optimized viral titration/serotypes, it is also quite feasible to infect only a subregion such as CA1, CA3, or DG areas only.

strong cooperativity and specific target gene selection. The co-occupancy state of various TF-binding sites and the specific regulation of each bound TF are likely to be at the heart of this complex regulatory mechanism, as demonstrated in the case of SARE element in the distal enhancer of the *Arc* gene (Kawashima et al., 2009) or in the CaRE element of the *BDNF* promoter (West et al., 2001). The modularity for many regulatory elements and the demonstration of multiple TF binding cassettes within critical enhancer/promoter regions now appear to be a dominant rule in most known enhancers of IEGs and in many genes across genomes (Kim et al., 2010; Rodriguez-Tornos, San Aniceto, Cubelos, & Nieto, 2013).

Expression of many activity-dependent TFs such as CREB, MEF2, and SRF is widespread across many brain regions. However, these must also possess differential roles in different brain regions and control distinct sets of downstream genes that are to be induced in each of these regions. How can such specificity be generated? As reviewed in many instances above, TcoFs, such as CRTC for CREB and TCF/MAL for SRF, are activity-dependently regulated in themselves and appear to add a layer of complexity to relatively simple regulations of TFs. Therefore, TcoFs may be crucial players that help define the specific contexts of activity-dependent gene induction: where, when, to what extent, and what sets of genes are induced during cognition (Fig. 2). Understanding how the specificity of

Table 2
Recent works on transcription factors/cofactors in neurons using a viral approach Sindbis virus has initially been used for forced expression of wild-type, active form and dominant negative forms of the TFs in behavior assessment, but was recently replaced by HSV, lentivirus and AAV for some reasons such as cell toxicity, safety and handling. In addition to *in vivo* application, lentivirus has also been effectively used in *in vitro* biochemical assays. Virus-mediated knockdown methods have been pioneered by (Hommel, Sears, Georgescu, Simmons, & DiLeone, 2003). For comprehensive lists of the viral constructs specifically for CREB research, refer to (Barco & Marie, 2011). Please note that this is not an exclusive survey and that the findings listed here do not cover all the contents of the reference papers.

Virus	Construct	Experiments	Findings	References
Studies to investigate the transcription factor/cofactor functions				
Sindbis virus	Sindbis-expression	*CREB* gain of function (behavior)	(CA1, DG) CREB ↑ → CFC ↑	Restivo et al. (2009) and Vetere, Marchetti, et al. (2011)
Lentivirus	Lenti-sh	*Npas4* knockdown (in culture)	Microarray identified Npas4 downstream genes	Lin et al. (2008)
	Lenti-sh	*Mef2* knockdown (in culture)	Microarray identified MEF2 downstream genes	Flavell et al. (2008)
	Lenti-sh	*NeuroD2* knockdown (morphology)	(CA3) NeuroD2 ↓ → spine density ↓	Wilke et al. (2012)
	Lenti-expression	*CREB* mutant expression (behavior)	(dHPC) CREB ↓ → CFC ↑	Kathirvelu, East, Hill, Smith, and Colombo, (2013)
	Lenti-expression	*CREB* expression (behavior)	(dStr) CREB ↑ → CFC ↑	Kathirvelu and Colombo (2013)
	Lenti-expression	*CRTC1* active form expression (morphology)	(CA1) CRTC1 ↑ → spine volume ↑	Nonaka et al. (2014)
HSV	HSV-expression	*CREB* expression study (behavior)	(BLA) CREB ↑ → Light FC ↑	Josselyn et al. (2001)
	HSV-expression	*CREB* expression study (behavior)	(LA) CREB ↑ → Auditory FC ↑	Han et al. (2007)
	HSV-Cre	*Npas4* knockout (behavior, in culture)	(CA3) Npas4 ↓ → CFC ↓ (culture) Npas4 ↓ → IEG↓	Ramamoorthi et al. (2011)
	HSV-expression	*Mef2* gain of function study (behavior)	(DG) MEF2 ↑ → WM↓, (LA) MEF2 ↑ → FC↓ (DG) MEF2 ↓ → WM ↑, (LA) MEF2 ↓ → FC ↑	Cole et al. (2012)
	HSV-expression	*CRTC1* gain of function study (behavior)	(DG) CRTC1 ↑ → CFC ↑	Sekeres et al. (2012)
AAV	AAV-sh	*Mef2* knockdown and gain of function (behavior, morphology)	(NAc) MEF2 ↓ → spine density ↑ (NAc) MEF2 ↑ → sensitization to cocaine ↑	Pulipparacharuvil et al. (2008)
	AAV-sh	*CREB* knockdown (behavior)	(NAc) CREB ↓ → cocaine CPP ↑	Green et al. (2010)
	AAV-Cre	*CBP* knockout (behavior)	(CA1) CBP ↓ → CFC ↓, spatial obj. recognition ↓	Barrett et al. (2011)
	AAV-sh	*Npas4* knockdown (behavior)	(LA) Npas4 ↓ → Auditory FC ↓	Ploski, Monsey, Nguyen, DiLeone, and Schafe (2011)
	scAAV	*MeCP2* knockout phenotype rescue (behavior)	(systemic) MeCP2 ↑ → motor task ↑	Garg et al. (2013) and Gadalla (2013)
	AAV-Cre	*Npas4* knockout (electrophysiology)	(CA1) Npas4 ↓ → mIPSC freq (after EE)↓	Bloodgood et al. (2013)
	AAV-sh, AAV-expression	*CRTC1* knockdown and gain of function (behavior)	(BLA) CRTC1 ↓ → FC ↓ (dHPC) CRTC1 ↑ → CFC ↑	Nonaka et al. (2014)
Studies expressing effectors in transcription factor/cofactor–modulated neurons				
HSV	HSV-dual expression	AlstR/ligand system	(LA) Auditory FC ↓	Zhou et al. (2009)
	HSV-dual expression	iDTR system	(LA) Auditory FC ↓	Han et al. (2009)
	HSV-dual expression	TRPV1 system	(LA) Auditory FC ↑	Kim et al. (2014)
	HSV-expression	dnKCNQ expression and DREADD (Gq) system (independent of HSV-CREB)	(LA) Auditory FC ↑	Yiu et al. (2014)

Abbreviations: AlstR, allatostatin G protein-coupled receptor; BLA, basolateral amygdala; CFC, contextual fear conditioning test; CPP, conditioned place preference; dHPC, dorsal hippocampus; DG, dentate gyrus; dnKCNQ, dominant-negative KCNQ (a type of K^+ channel); DREADD, Designer Receptors Exclusively Activated by Designer Drugs; dStr, dorsal striatum; EE, enriched environment; FC, fear conditioning test; iDTR, Cre-inducible diphtheria toxin receptor; LA, Lateral amygdala; mIPSC, miniature inhibitory postsynaptic current; NAc, nucleus accumbens; obj. recognition, object recognition; sh, short hairpin; scAAV, self-complementary AAV; TRPV1, transient receptor potential vanilloid receptor 1; WM, watermaze task.

activity-dependent gene expression is generated in the brain is critical in dissecting fine-tuned information processing during many different cognitive behaviors. The interaction and combinatorial regulation via multiple regulatory elements within promoters/enhancers, as mentioned above, will also work as part of a multi-layered regulatory mechanism by which the genome dictates when and where the activity-induced genes are to be expressed.

One large caveat associated with the systematic interrogation of activity-dependent TF functions is the fact that task-related activity occurs in sparsely distributed neurons in large brain areas during cognitive processes, perhaps with the exception of the cases of massive population firing in pathological cases such as epileptic seizures. Therefore, one needs to identify task-dependent active neuronal ensembles to achieve better understanding of the link between TF/TcoF, IEGs and the behavior. To meet such growing demands, IEG promoters have of late been optimized to allow viral application with a view to marking those sparse neuronal populations (Kawashima et al., 2013; Kawashima et al., 2014). Such advance may further facilitate systematic investigation of active ensembles, through transcriptome analysis, and calcium imaging using activity-sensors such as a genetically encoded calcium indicator, GCaMP6 (Chen et al., 2013).

One outstanding question that remains to be addressed in future studies is to tease apart the distinctive roles of TFs of seemingly opposite roles in cognitive function, which nonetheless appears to target similar sets of IEGs. As detailed above, the cooperativity of many of these factors (e.g. CREB-MeCP2 and CREB-DREAM) may not only be controlled by activity but may also depend on the precise brain location. Comprehensive studies using high throughput ChIP-seq/ChIP-chip of many of the activity-

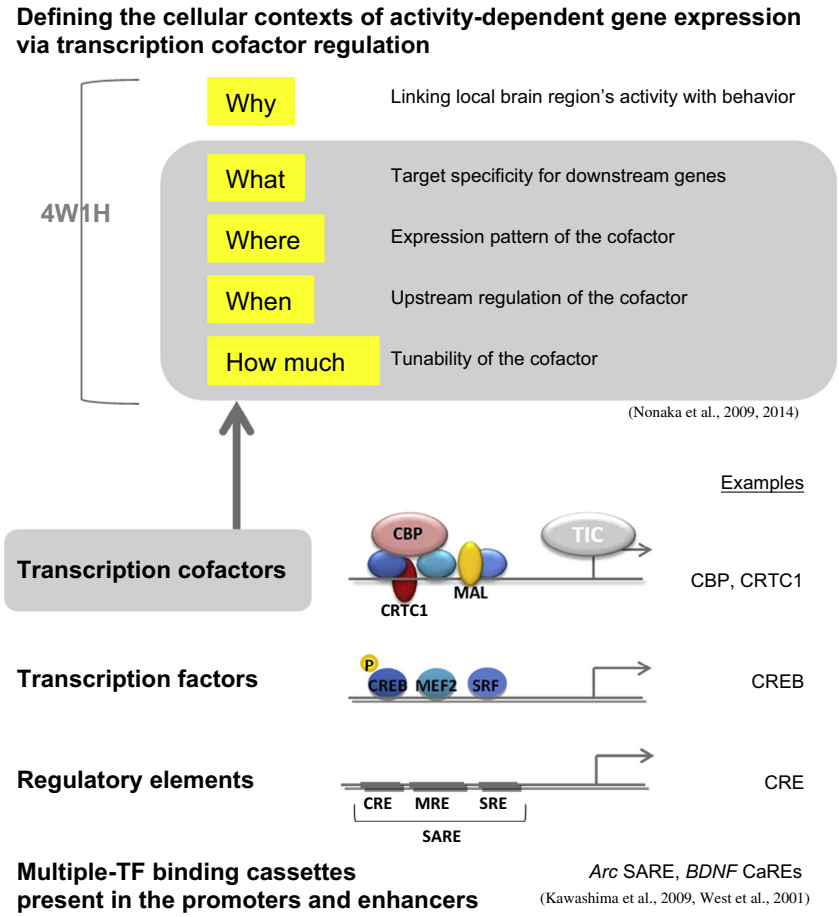

Fig. 2. Transcription factors/co-factors enables the regulation of 4W1H of targeted gene expression. TFs bind target recognition DNA sequences within regulatory sequences contained in the promoter and enhancers. Some transcription factors (TFs) are specifically expressed in some specific cell types and at selective developmental stages. However, many TFs are ubiquitously expressed in the brain, and are known to be critical for many physiological brain functions. Therefore, there must be another level of regulations to define the contexts for gene expression; where and when, how much of what set of genes are expressed. Here we propose a model in which the transcription cofactors (TcoFs) help achieve a substantial degree of brain-region specificity, downstream gene set specificity, and gain control for gene expression. Multiple-transcription factor (TF) binding modules, as discovered in some IEG promoters/enhancers, may additionally underlie controlled cooperativity and combinatorial effects, through interactions of transcription factors and cofactors. TIC, transcription initiation complex.

dependent TFs/TcoFs, and RNA-Seq analyses of neurons constituting active ensembles in specific regions of the brain, during several stages of behavior tasks, will enlighten the molecular basis of this question. It will be also critical to reveal the region-specific availability of upstream signalling cascades and cofactors that participate in such regulation. Together, we anticipate that these combinatorial approaches will contribute to enhance our understanding of molecular pathology underlying cognitive and learning disabilities that are based on dysregulation of TFs/TcoFs, such as Rett syndrome and Rubinstein-Taybi syndrome (Cohen & Greenberg, 2008; Ebert & Greenberg, 2013).

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